Journal of Infection and Public Health (2015) 8, 634—641

http://www.elsevier.com/locate/jiph

SHORT COMMUNICATION

Epidemiology of necrotizing infection caused by *Staphylococcus aureus* and *Streptococcus pyogenes* at an Iowa hospital

Dipendra Thapaliya[a,b,1], Ashley M. O'Brien[a,b], Shylo E. Wardyn[a,b], Tara C. Smith[a,b,*,1]

[a] *Department of Epidemiology, University of Iowa College of Public Health, Iowa City, IA, USA*
[b] *Center for Emerging Infectious Diseases, University of Iowa College of Public Health, Iowa City, IA, USA*

Received 2 February 2015; received in revised form 28 May 2015; accepted 12 June 2015

KEYWORDS
Staphylococcus aureus;
Streptococcus pyogenes;
Necrotizing fasciitis;
Epidemiology;
Infection

Summary The present study was performed to characterize the epidemiology of necrotizing soft tissue infection caused by *Streptococcus pyogenes* ($n = 14$) and *Staphylococcus aureus* ($n = 14$) isolates collected at the University of Iowa Hospitals and Clinics. An additional 9 *S. pyogenes* isolates were collected from patients being treated for mild respiratory infections and served as a comparison sample in the analysis. Patient data corresponding to the isolates ($n = 37$) were also collected in order to identify risk factors or comorbid conditions possibly correlated with necrotizing fasciitis (NF). The prevalence of methicillin-resistant *S. aureus* among the study isolates was 35.7% (5/14), and the prevalence of the Panton—Valentine leukocidin (PVL) gene was 57% (8/14). The *S. pyogenes* NF (wound) isolates ($n = 14$) belonged to 10 different *emm* types, none of which appeared to be associated with more severe disease when compared to the milder infection (throat) samples ($n = 9$). Comorbid conditions such as diabetes and cardiovascular disease were significantly associated with NF. The results indicate that there may be a high prevalence of the PVL virulence factor in NF infections and that *spa* type t008 may be responsible for the increasing incidence of *S. aureus* NF infections in Iowa.

* Corresponding author at: 750 Hilltop Drive, Lowry Hall, Kent, OH 44242, USA. Tel.: +1 330 672 3946.
E-mail address: tsmit176@kent.edu (T.C. Smith).
[1] Present address: Department of Biostatistics, Environmental Health Sciences, & Epidemiology, College of Public Health, Kent State University, Kent, OH, USA.

http://dx.doi.org/10.1016/j.jiph.2015.06.003
1876-0341/© 2015 King Saud Bin Abdulaziz University for Health Sciences. Published by Elsevier Limited. All rights reserved.

Introduction

In the United States, the incidence of necrotizing infections is approximately 0.04 cases per 1000 persons-years [1]; nevertheless, the disease is often characterized by its rapid progression and high mortality rate, which is estimated to range from 25% to 35% [1,2], though some studies have reported a mortality rate as high as 50% [3]. Most necrotizing infection cases are caused by *Streptococcus pyogenes*; however, the incidence of necrotizing fasciitis (NF) with *Staphylococcus aureus* identified as the primary pathogen is on the rise [4,5].

To date, little is known about the pathogenicity of necrotizing infections, primarily regarding molecular characterization and virulence factors, as well as host factors that may be correlated with severe necrotizing infections. Three notable virulence factors associated with *S. pyogenes* infection include *emm* type, mutations in the *covR*/*covS* system, and the presence of superantigens. Previous research has indicated that certain *emm* types may be responsible for necrotizing *S. pyogenes* infections, particularly *emm* types 1, 3, and 12 [6—8].

A key virulence factor of interest for *S. aureus* infections is the presence of the Panton—Valentine leukocidin (PVL) gene. Of particular interest, two previous studies performed molecular typing on subsets of methicillin-resistant *S. aureus* (MRSA) isolates causing NF and reported the presence of the PVL gene in 100% of the samples (5/5) [4,9].

Along with molecular characterization, numerous studies have investigated the association between certain host factors and an increased risk of NF. Studies examining patient medical histories have identified a multitude of factors, including prior trauma, surgery, nonsteroidal anti-inflammatory drug use, burns, chronic alcohol consumption, immunosuppressive drug use, cancer, diabetes, obesity, and renal disease, among others, that may be correlated with NF [10—12]. However, studies also have reported the occurrence of necrotizing infections in patients with no known risk factors or comorbid conditions [4,11].

The previously described findings demonstrate the need for further research regarding the etiology of NF. The goals of our study were to determine the molecular characterization of NF caused by *S. pyogenes* and *S. aureus*, including *emm* type (*S. pyogenes*), *spa* type, and the presence of the PVL and *mecA* genes (*S. aureus*), and to identify whether specific host factors are correlated with severe necrotizing infections among patients at the University of Iowa Hospitals and Clinics.

Materials and methods

Study isolates ($n = 38$) were collected and stored at the University of Iowa Hospitals and Clinics Pathology Department between January 2011 and September 2012. Fourteen *S. aureus* and 14 *S. pyogenes* samples were collected from cases of necrotizing infection; the remaining 10 *S. pyogenes* isolates were obtained from throat cultures collected from patients being treated for mild respiratory infections and served as a comparison in the analysis. Isolates were attained from the Pathology Department in October 2012 following IRB approval, and were analyzed at the Center for Emerging Infectious Diseases with molecular typing.

Genomic DNA extraction was performed using the Wizard Genomic DNA preparation kit (Promega, WI). Polymerase chain reaction (PCR) was performed to detect *mecA* and PVL genes (*lukS*, *lukF*) present in the *S. aureus* isolates [13,14]. The staphylococcus protein A (*spa*) gene was amplified using SpaF (5′-GAACAA-CGTAACGGCTTCATCC-3′) and 1514R (5′-CAGCAGTAGTGCCGTTTGCCT-3′), as previously described [15,16]; *emm* typing was carried out for all *S. pyogenes* isolates [17], and 16s rRNA PCR was performed with all isolates to confirm the species [18]. Upon completion of 16s rRNA PCR, 1 isolate among the *S. pyogenes* throat samples was found to belong to the *Streptococcus parasanguinis* species, and was subsequently excluded from further analysis, leaving 9 throat infection isolates. Multilocus sequence typing was completed on all but 1 study isolate [19].

All *S. aureus* and 23 *S. pyogenes* isolates were tested for antibiotic susceptibility by using the VITEK 2 System (bioMérieux). We used the AST-GP71 and AST-ST01 cards of the VITEK 2 System for the antibiotic susceptibility testing of *S. aureus* and *S. pyogenes*, respectively. *S. aureus* isolates were tested for susceptibility to benzylpenicillin, oxacillin, tetracycline, erythromycin, ciprofloxacin, moxifloxacin, minocycline, clindamycin, trimethoprim—sulfamethoxazole, quinupristin/dalfopristin, gentamicin, levofloxacin, linezolid, daptomycin, vancomycin, rifampicin, minocycline, tigecycline, and nitrofurantoin. *S. pyogenes* isolates were tested for susceptibility to benzylpenicillin, ampicillin, cefotaxime, ceftriaxone, tetracycline, erythromycin, clindamycin, trimethoprim—sulfamethoxazole, levofloxacin, linezolid, and vancomycin. Isolates showing intermediate levels of susceptibility were classified as resistant. *S. aureus* isolates that were resistant to 3 or more classes of antimicrobials or that were

resistant to oxacillin were considered multidrug resistant [20].

We retrieved patient data from the medical records of the University of Iowa Hospitals and Clinics and linked patients' medical record numbers to the bacterial specimens for the purposes of investigating host factors that may be associated with necrotizing infection. Patients' demographics, clinical data, comorbid conditions, outcome variables, and specimen-specific data were extracted and entered into the database. Analysis of patient data was conducted using SAS statistical software (version 9.3, SAS Institute Inc., Cary, NC).

Results

Molecular typing revealed 9 different *spa* types among the *S. aureus* study isolates, with the only repeat type being t008 (43%; $n = 6$). The prevalence of MRSA in the study sample was 35.7% (5/14). All of the MRSA isolates were *spa* type t008. Fifty-seven percent (8/14) of the *S. aureus* isolates harbored the PVL gene, while all MRSA isolates harbored the PVL genes. Most of the *S. aureus* isolates (86%; 12/14) contained a functional *agr*.

A total of 7 different sequence types were detected among 14 necrotizing infection *S. aureus* isolates. Among *S. aureus* isolates, ST8 was the most common sequence type identified (46.1%; 6/13), followed by ST188 (15.4%; 2/13). No other sequence types (38.5%; 5/13) were found to be repeated (Table 1a).

Among the *S. pyogenes* isolates, there were 14 different *emm* types represented: 17.4% were *emm*12 ($n = 4$), 13% were *emm*11 ($n = 3$), 13% were *emm*89 ($n = 3$), and 8.7% were *emm*77 ($n = 2$) (Table 1b). A total of 15 different sequence types were detected among the 14 necrotizing infection and 9 throat infection *S. pyogenes* isolates. ST36 was the most common sequence type identified (17%; 4/23), followed by ST678 (13%; 3/23), ST101 (13%; 3/23), and ST63 (9%; 2/23). No other sequence types (48%; 11/23) were found to be repeated.

Among 14 *S. aureus* isolates, oxacillin resistance was observed in 35.7% (5/14). Four isolates (28.6%) were resistant to ciprofloxacin; 7 (50%) were resistant to erythromycin; 2 (14.3%) were resistant to clindamycin; 3 (21.4%) were resistant to levofloxacin; and all 14 (100%) were resistant to benzylpenicillin. Eight isolates (57%) were multidrug resistant *S. aureus*. Among 23 *S. pyogenes* isolates, 7 isolates (30.4%) were resistant to erythromycin; 1 (4.3%) was resistant to ciprofloxacin; 8 (34.8%) were resistant to clindamycin; 1 (4.3%) was resistant to ceftriaxone; 7 (30.4%) were resistant to tetracycline; and 1 (4.3%) was resistant to cefotaxime.

Sixty-nine percent (9/13) and 31% (4/13) of the NF patients were male and female, respectively. Sixty-nine percent of the patients (9/13) were white, and 31% (4/13) were African American. The median age of the patients was 48 years ($n = 13$; mean, 42.69; standard deviation, 12.65; range, 21—61) (Table 2).

The median length of hospitalization was 10 days ($n = 13$; mean, 12.4; standard deviation, 10.2; range, 1—38 days). The majority of patients (92%; 12/13) diagnosed with NF underwent surgery. The sites of NF included the extremity (left foot: 2; right foot: 1; left ankle: 1; left lower leg: 1; right lower leg: 2) in 7 patients (53.8%), trunk (left upper back and back trunk) in 2 patients (15.4%), perineum in 3 patients (23.1%), and both trunk and extremity in 1 patient (7.7%) (Table 2).

Table 1a Molecular characteristics of *S. aureus*.

Isolate ID	Source	*mecA*	PVL	*spa*	AST	MLST	*agr* phenotype
1	Necrotizing infection	—	—	t189	P, C, E, Cl	ST188	Functional
2	Necrotizing infection	—	—	t1109	P, O, C, L, E	ST97	Functional
3	Necrotizing infection	—	—	t701	P, E, Cl	ST6	Functional
4	Necrotizing infection	—	+	t008	P, E	ST8	Functional
5	Necrotizing infection	—	—	t088	P	ST5	Functional
6	Necrotizing infection	—	—	t8870	P	ST188	Functional
7	Necrotizing infection	+	+	t008	P	ST8	Functional
8	Necrotizing infection	—	—	t5051	P	ST45	Functional
9	Necrotizing infection	+	+	t008	P, T, TS	ST8	Functional
10	Necrotizing infection	+	+	t008	P, O	ST8	Functional
11	Necrotizing infection	+	+	t008	P, O, C, L, E	ST8	Functional
12	Necrotizing infection	—	+	t094	P	ST15	Dysfunctional
13	Necrotizing infection	+	+	t008	P, O, C, L, E	ST8	Functional
14	Necrotizing infection	—	+	t10075	P, O, E	ST152	Dysfunctional

Table 1b Molecular characteristics of *S. pyogenes*.

Isolate ID	Source	*EMM* type	AST	MLST
15	Necrotizing infection	*emm*1.0	—	ST28
16	Necrotizing infection	*emm*6.4	—	ST382
17	Necrotizing infection	*emm*12.0	—	ST36
18	Necrotizing infection	*emm*87.0	—	ST62
19	Necrotizing infection	*emm*102.2	C, CT, E, Cl	ST60
20	Necrotizing infection	*emm*28.4	—	ST52
21	Necrotizing infection	*emm*92.0	E, Cl, T	ST82
22	Necrotizing infection	*emm*12.0	E, Cl	ST36
23	Necrotizing infection	*emm*103.0	CT, CX	ST327
24	Necrotizing infection	*emm*11.0	E, Cl, T	ST678
25	Necrotizing infection	*emm*2.0	—	ST55
26	Necrotizing infection	*emm*89.0	—	ST101
27	Necrotizing infection	st106M.5	T	ST53
28	Necrotizing infection	*emm*89.0	—	ST101
29	Respiratory infection	*emm*3.1	Cl	ST15
30	Respiratory infection	*emm*89.0	—	ST101
31	Respiratory infection	*emm*12.0	—	ST36
32	Respiratory infection	*emm*77.0	T	ST63
33	Respiratory infection	*emm*11.0	E, Cl, T	ST678
34	Respiratory infection	*emm*77.0	T	ST63
35	Respiratory infection	*emm*4.0	—	ST39
36	Respiratory infection	*emm*12.0	E, Cl	ST36
37	Respiratory infection	*emm*11.0	E, Cl, T	ST678

P, benzylpenicillin; C, ciprofloxacin; E, erythromycin; Cl, clindamycin; O, oxacillin; T, tetracycline; TS, trimethoprim/sulfamethoxazole; CT, ceftriaxone; CX, cefotaxime; —, susceptible to all antibiotics tested.

A majority of the patients (84.6%; 11/13) had at least 1 comorbid condition or risk factor; the most common comorbid condition was obesity (69.2%, 9/13), followed by cardiovascular disease (61.5%; 8/13), diabetes (61.5%; 8/13), pulmonary disease (30.8%; 4/13), neurological disorders (peripheral neuropathy, schizophrenia, anxiety, depression, panic attack, attention-deficit hyperactive disorder) (23%; 3/13), cancer (15.4%; 2/13), malnutrition (7.7%; 1/13), and illicit drug use (7.7%; 1/13). Two patients (15.4%; 2/13) had no serious comorbid conditions or risk factors (Table 2). There was a higher incidence of NF among men (69.2%; 9/13) compared to women (30.7%; 4/13). Similarly, white patients were more affected (69.2%; 9/13) than African—American patients (30.7%; 4/13). No other race or ethnicity was reported. Sixty-nine percent (9/13) and 31% (4/13) of the patients had monomicrobial and polymicrobial infections, respectively (Table 2).

Discussion

More than 100 distinct molecular types of *S. pyogenes* have been identified on the basis of M protein, which is encoded by the *emm* gene [21]. It is hypothesized that some *emm* types may be more pathogenic than others [22]. In this study, only 2 *emm* types (*emm*1.0 and *emm*87.0) were found in NF cases. All other NF cases diagnosed in this study were caused by *S. aureus*.

In recent decades, the incidence of life-threatening invasive infection of community-associated MRSA (CA-MRSA) is increasing in many parts of the world [4]. MRSA has been identified as the most commonly isolated pathogen from patients with skin and soft tissue infections presenting in U.S. emergency departments [23]. In our study, 38.5% (5/13) of the NF infections were caused by CA-MRSA. The results of our study are consistent with a nationwide surveillance study that found a high prevalence of MRSA (51%; 2093/4131) in clinically significant *S. aureus* isolates [24]. In their study, USA300 was the most common strain ($n = 1269$; 61%), and two-thirds of USA300 isolates originated from wound and skin infections. Sixty-four percent of the MRSA isolates harbored PVL genes [24]. This is in contrast to a study of *S. aureus* asymptomatic carriage conducted in the same area of Iowa as the current study, in which only 5.2% of isolates collected were positive for PVL [25], suggesting that invasive infections are enriched for PVL-positive isolates. Another study conducted in Los Angeles, California, reported 29% (9/31) of cases of NF were caused by CA-MRSA [4].

Table 2 Characteristics of the patients.

Isolate ID	Age	Sex	Race or ethnicity	Organisms in positive culture	Microbial status	Diagnosis	Duration of hospitalization (days)	Surgery	Site of infection	Comorbid conditions
1 & 15	49	F	White	SA	p	NF	16	Yes	Left lower leg	DM, CVD, OBS
2	31	M	American Indian	SA	M	CL	2	No	Lower abdomen	None
3	61	M	African American	SA & SP	P	NF	38	Yes	Left foot	CVD, DM, PD, peripheral neuropathy, OBS
4	57	M	White	SA	M	NF[b]	6	Yes	Right foot	CVD, DM, cancer, OBS
5	51	M	White	SA	M	NF	1	No	Perineal	CVD, DM, PD, OBS illicit drug use
6	44	M	White	SA	M	OST	5	Yes	Right foot	CVD, DM, PD, GID, peripheral neuropathy
7	51	F	African American	SA	M	NF	15	Yes	Left upper back	CVD, DM, PD, OBS, Schizophrenia
8	52		White	SA	P	NF	4	Yes	Right lower leg	CVD
9	32	M	White	SA (MRSA)	M	NF	25	Yes	Perineal	OBS
10	21	F	White	SA	M	NF	7	Yes	Left ankle	None
11	23	F	White	SA (MRSA)	M	NF	13	Yes	Trunk & left upper arm	DM
12	35	M	White	SA	M	NF	17	Yes	Right foot	CVD, DM, PD, cancer, OBS, malnutrition, panic attack
13	36	M	White	SA (MRSA)	M	NF	4	Yes	Right lower leg	OBS
14	48	M	African American	SA (MRSA)[a]	P	NF	10	Yes	Back trunk	CVD, DM, OBS
16	32	M	White	SP	M	CL	1	Yes	Right forearm	Illicit drug use, depression, anxiety, ADHD
17	51	F	White	SP	M	BA	1	Yes	Left breast	None

18	39	M	African American	SP	M	NF	5	Yes	Perineal	None
19	21	F	Hispanic/ multiracial	SP	M	AP & I	1	No	Neck (pharyngitis)/extremities (impetigo)	None
20	39	M	Non-Hispanic/ multiracial	SP	M	PRT	5	Yes	Left hip	None
21	21	M	Unknown	SP	M	CRB & AP	1	No	Neck & right lower leg	None
22	44	M	White	SA (MRSA) & SP	P	CL	3	No	Left ankle	OBS
23	23	M	White	SP	M	CL	1	No	Perirectal	None
24	41	M	White	SP	M	SI	1	No	Back/left shoulder & arm	GID
25	44	M	White	SA & SP	P	CL	5	Yes	Left forearm	GID, ADHD
26	56	M	White	SA & SP	P	PVD	1	Yes	Left foot	CVD, malnutrition, GID, PTSD, depression
27	48	F	White	SP	M	LCR	1	No	Right hand	None
28	38	F	White	SP	M	CL	1	No	Left elbow	GID

Abbreviations used: SP, *Streptococcus pyogenes*; SA, *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*; M, mono-microbial; P, poly-microbial; NF, necrotizing fasciitis; CL, cellulitis; OST, osteomyelitis; BA, breast abscess; AP, acute pharyngitis; FVR, fever/fatigue; SI, skin infection; I, impetigo; LCR, hand laceration; PRT, peritonitis; DM, diabetes mellitus, CVD, cardiovascular disease; PD, pulmonary disease; GID, gastrointestinal disease; ADHD, attention deficit hyperactivity disorder; PTSD, post-traumatic stress disorder.

[a] *Klebsiella pneumonia* was also isolated from wound.

[b] Secondary to diabetic cellulitis.

In contrast to the previous report of high prevalence of monomicrobial MRSA in wound cultures (86%; 12/14), only 3 cases of NF (23%; 3/13) in our study were caused by monomicrobial CA-MRSA [4]. However, consistent with a previous study in which all of the tested isolates ($n = 5$) were ST 8- and PVL-positive [4], ST8 (USA300/t008) was the most common sequence type in our study, and more than half of our *S. aureus* isolates and all MRSA isolates harbored the PVL gene. Although CA-MRSA USA300 has been well documented in many outbreaks of soft tissue infections, the findings of this study, those of another study that documented 16.7% of CA-MRSA NF cases were USA 300 [9], and those of a study by Miller et al. [4] demonstrate the rising incidence of NF caused by CA-MRSA in the United States. The typical mortality rate of NF is about 33% [4]. However, similar to a previous study [4], none of the NF patients in our study died; this may be due to differences in care, in study population, or in the bacteriology of the NF infections in this hospital. This finding also suggests the possibility that the strain of CA-MRSA causing NF in our study population may be less virulent than NF caused by other organisms.

Previous studies have documented several risk factors for necrotizing soft tissue infection, including diabetes mellitus, intravenous drug abuse, peripheral vascular disease, obesity, malnutrition, blunt or penetrating trauma, alcohol abuse, surgical incisions, chicken pox, vesicles, and immunosuppression [1,2]. The majority of the patients of this study had at least 1 comorbid condition or risk factor. The *S. pyogenes* study isolates belonged to 14 different *emm* types, with no specific *emm* type being over-represented among the necrotizing infection cases versus the mild respiratory infection controls; however, no broad conclusions can be drawn due to the small sample size of NF cases caused by *S. pyogenes*.

The results of this study indicate that several *spa* and *emm* types are responsible for NF cases presenting at the University of Iowa Hospitals and Clinics. All of the MRSA NF isolates in the study were *spa* type t008, suggesting this strain of *S. aureus* may be associated with increased disease severity in Iowa. Our study has several limitations. Though this study was prospective in design [11], not all microbial cultures were banked for later analysis. As such, our study included convenience samples of both *S. aureus* and *S. pyogenes* in 1 hospital, thus limiting the generalizability of results. However, this study provides insights into the molecular characteristics of *S. aureus* and *S. pyogenes* causing NF at University of Iowa Hospitals and Clinics. Future research should focus on analyzing samples from all NF cases within a facility, and analyzing molecular characteristics of all causative agents.

Funding

Center for Emerging Infectious Diseases, University of Iowa.

Conflict of interest

None declared.

Ethical approval

The University of Iowa IRB evaluated this project and determined that it did not qualify as human subjects research.

References

[1] Sarani B, Strong M, Pascual J, Schwab CW. Necrotizing fasciitis: current concepts and review of the literature. J Am Coll Surg 2009;208:279—88.
[2] Singh G, Chawla S. Aggressiveness—the key to a successful outcome in necrotizing soft tissue infection. Med J Forces India 2003;59:21—4.
[3] Freischlag JA, Ajalat G, Busuttil RW. Treatment of necrotizing soft tissue infections: the need for a new approach. Am J Surg 1985;149:751—5.
[4] Miller LG, Perdreau-Remington F, Rieg G, Mehdi S, Perlroth J, Bayer AS, et al. Necrotizing fasciitis caused by community-associated methicillin-resistant *Staphylococcus aureus* in Los Angeles. N Engl J Med 2005;352:1445—53.
[5] Lee TC, Carrick MM, Scott BG, Hodges JC, Pham HQ. Incidence and clinical characteristics of methicillin-resistant *Staphylococcus aureus* necrotizing fasciitis in a large urban hospital. Am J Surg 2007;194:809—13.
[6] Luca-Harari B, Ekelund K, van der Linden M, Staum-Kaltoft M, Hammerum AM, Jasir A. Clinical and epidemiological aspects of invasive *Streptococcus pyogenes* infections in Denmark during 2003 and 2004. J Clin Microbiol 2008;46:79—86.
[7] Luca-Harari B, Darenberg J, Neal S, Siljander T, Strakova L, Tanna A, et al. Clinical and microbiological characteristics of severe *Streptococcus pyogenes* disease in Europe. J Clin Microbiol 2009;47:1155—65.
[8] O'Loughlin RE, Roberson A, Cieslak PR, Lynfield R, Gershman K, Craig A, et al. The epidemiology of invasive group A streptococcal infection and potential vaccine implications: United States, 2000—2004. Clin Infect Dis 2007;45:853—62.
[9] Young LM, Price CS. Community-acquired methicillin-resistant *Staphylococcus aureus* emerging as an important cause of necrotizing fasciitis. Surg Infect 2008;9:469—74.
[10] Childers BJ, Potyondy LD, Nachreiner R, Rogers FR, Childers ER, Oberg KC, et al. Necrotizing fasciitis: a fourteen-year retrospective study of 163 consecutive patients. Am Surgeon 2002;68:109—16.

[11] Bernal NP, Latenser BA, Born JM, Liao J. Trends in 393 necrotizing acute soft tissue infection patients 2000—2008. Burns 2012;38:252—60.
[12] Tunovic E, Gawaziuk J, Bzura T, Embil J, Esmail A, Logsetty S. Necrotizing fasciitis: a six-year experience. J Burn Care Res 2012;33:93—100.
[13] Boşgelmez-Tınaz G, Ulusoy S, Arıdoğan B, Coşkun-Arı F. Evaluation of different methods to detect oxacillin resistance in *Staphylococcus aureus* and their clinical laboratory utility. Eur J Clin Microbiol Infect Dis 2006;25:410—2.
[14] Lina G, Piémont Y, Godail-Gamot F, Bes M, Peter M-O, Gauduchon V, et al. Involvement of Panton—Valentine leukocidin—producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin Infect Dis 1999;29:1128—32.
[15] Shopsin B, Gomez M, Montgomery SO, Smith DH, Waddington M, Dodge DE, et al. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. J Clin Microbiol 1999;37: 3556—63.
[16] Koreen L, Ramaswamy SV, Graviss EA, Naidich S, Musser JM, Kreiswirth BN. spa typing method for discriminating among *Staphylococcus aureus* isolates: implications for use of a single marker to detect genetic micro-and macrovariation. J Clin Microbiol 2004;42:792—9.
[17] Centers for Disease Control and Prevention. Protocol for emm typing. Atlanta, GA: Centers for Disease Control and Prevention; 2008.
[18] Bosshard P, Abels S, Zbinden R, Böttger E, Altwegg M. Ribosomal DNA sequencing for identification of aerobic gram-positive rods in the clinical laboratory (an 18-month evaluation). J Clin Microbiol 2003;41:4134—40.
[19] Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol 2000;38:1008—15.
[20] Magiorakos AP, Srinivasan A, Carey R, Carmeli Y, Falagas M, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268—81.
[21] Cunningham MW. Pathogenesis of group A streptococcal infections. Clin Microbiol Rev 2000;13:470—511.
[22] Bessen DE, Izzo MW, Fiorentino TR, Caringal RM, Hollingshead SK, Beall B. Genetic linkage of exotoxin alleles and emm gene markers for tissue tropism in group A streptococci. J Infect Dis 1999;179:627—36.
[23] Moran GJ, Krishnadasan A, Gorwitz RJ, Fosheim GE, McDougal LK, Carey RB, et al. Methicillin-resistant *S. aureus* infections among patients in the emergency department. N Engl J Med 2006;355:666—74.
[24] Diekema DJ, Richter SS, Heilmann KP, Dohrn CL, Riahi F, Tendolkar S, et al. Continued emergence of USA300 methicillin-resistant *Staphylococcus aureus* in the United States: results from a nationwide surveillance study. Infect Control Hosp Epidemiol 2014;35:285—92.
[25] Moritz ED, Hanson BM, Kates AE, Smith TC. Molecular characteristics of *Staphylococcus aureus* isolated from employees, children, and environmental surfaces in Iowa child daycare facilities. Am J Infect Control 2015;43:482—8.